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PAPER

Influence of home characteristics on airborne and dustborne endotoxin and β-D-glucan

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The aim of this study was to assess the associations between airborne and dustborne microbial contaminants (endotoxin and β-D-glucan) and estimate the effects of home characteristics on exposure levels of these microbial contaminants. Endotoxin and β-D-glucan concentrations in airborne inhalable particles, airborne PM1 (<1 μm) and vacuumed dust from 184 residential homes were determined using specific *Limulus* amebocyte assays. Home characteristics were recorded by visual inspection and questionnaires. Linear regression and correlation analyses were performed. Inhalable endotoxin correlated with dust endotoxin ($r = 0.34$, $p < 0.001$) and PM1 endotoxin ($r = 0.33$, $p < 0.001$). Inhalable β-D-glucan correlated with dust β-D-glucan ($r = 0.18$, $p < 0.01$), but not with PM1 β-D-glucan. Significant correlation was also found between PM1 and dust concentrations for endotoxin ($r = 0.26$, $p < 0.001$), but not for β-D-glucan. Multivariate regression analyses showed only one significant association between airborne contaminants and environmental characteristics: inhalable β-D-glucan was positively associated with relative humidity with an effect size (*change in the dependent variable corresponding to a unit increase in the independent variable*) of 2.32 and $p < 0.05$. In contrast, several associations were found between dust concentrations and environmental characteristics. Dust endotoxin was positively associated with temperature (2.87, $p < 0.01$) and number of inhabitants (2.76, $p < 0.01$), whereas dust β-D-glucan was inversely associated with the presence of dogs (−2.24, $p < 0.05$) and carpet (−3.05, $p < 0.01$) in the home. In conclusion, dustborne contaminants were more strongly affected by home characteristics than airborne contaminants. Furthermore, even though statistically significant, the correlations between airborne and dustborne contaminants were weak. This indicates that airborne concentrations cannot be reliably predicted based on dustborne concentrations.

Introduction

Exposure to microbial contaminants in indoor environments has been assessed in various investigations by using air or dust sampling. Cultivable microorganisms, microbial spores or their cell wall components as well as microbial toxins or enzymes produced by them have been commonly used for qualitative and quantitative assessment of microbial contamination of indoor air.[1–4] The indoor bioaerosol transport is enhanced by non-biological solid components, including those in dust that may act as the vectors for biological contaminants penetrating into the lungs.[5] An important factor affecting the aerosolization of microbial contaminants from dust in homes is mechanical disturbance,[6–8] which is generally dependent on the presence and indoor activities of occupants and pets.[9] The aerosolization of biological particles from surfaces is also affected by relative

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Environmental impact

Environmental exposures to microbial contaminants can be detrimental to human health. Traditional methods of exposure assessment have been limited to sampling of settled dust particles or non-size-selective sampling of airborne microbial particles. Samples collected through these methods convey little information about the probability of these particles reaching the human airways and causing respiratory diseases. In this study, we have investigated correlations of microbial contaminants in settled dust with those size selectively collected from the air. Weak to moderate correlations between airborne and dustborne exposure variables were found. This emphasizes the need for size-selective assessment of airborne microbial particles in order to determine the exposure risk for different respiratory diseases conferred through these airborne microbial contaminants.

humidity, temperature, ventilation, source of dust and type of flooring.[10–12] These factors, along with season and housing conditions (*e.g.*, water intrusion) and the presence of carpeted floors, presence of dogs, and occupant density have been implicated in affecting the concentrations of indoor microbial contaminants.[13–17] However, the associations of microbial contaminants with environmental factors identified in previous studies have been inconsistent.[13,18–20] The variations in findings among different reports suggest that various factors, either environmental or inherent to the microbial contaminants, interact in a complex way. These multiple interactions can affect the concentrations of the microbial contaminants measured in airborne particles or in dust. Additionally, factors affecting microbial growth may differ from those affecting aerosolization of microorganisms or their cellular components.[21]

As aerosolization and indoor transport of airborne particles are largely governed by environmental conditions, the particle concentration can vary from day to day within the same home. Consequently, the concentrations of airborne microbial contaminants represent transient and variable exposure levels.[22,23] On the contrary, concentrations of microbial particles in settled dust are believed to represent an increasing accumulation of microbial particles and are often used as surrogate for measures of chronic exposures to these agents.[24] Only a few of the previous studies have focused on the correlation between dust-borne and airborne contaminants. Endotoxin concentration in dust is known to correlate weakly with that in airborne particles.[13] Dust β-D-glucan concentration in indoor dust has also been poorly correlated with that in air.[25]

Exposure to airborne microbial particles has been associated with infectious or allergic respiratory diseases.[26] The ability of the airborne particles to reach and settle in the various parts of the respiratory system is a function of their aerodynamic diameter.[27–30] In particular, exposures to inhalable particles (particulate matter with median aerodynamic diameter, d_a, less than 100 μm) and thoracic particles (d_a < 10 μm) have been considered as important determinants of human health outcomes.[31] With gradual awareness about the ability of fine particles (PM1, d_a < 1.0 μm) to reach and impact in the distal airways, the focus of studies dealing with health effects associated with exposure to airborne microbial particles has shifted towards smaller size fractions.[32,33] Because of high penetration and retention, the sub-micrometre particles can trigger inflammation of airways or alteration in immune response.[34] Although there are several reports on the concentrations of PM1 endotoxin and β-D-glucan in homes,[4,35–38] the correlations of PM1 microbial contaminants with those in inhalable particles or in settled dust have not been previously studied. In addition, the influence of various home characteristics on PM1 concentrations is currently equivocal.

In this study, we quantified bacterial and fungal contaminants in PM1 and inhalable airborne particles as well as in settled dust inside homes. Subsequently, we investigated associations of PM1 contaminants with those measured in inhalable particles and dust. Endotoxin (lipopolysaccharide component in Gram-negative bacterial cell walls) was selected as a surrogate for the measurement of bacterial contamination; (1 → 3)-β-D-glucan (β-D-glucan; fungal cell wall component) served as a surrogate of fungal contamination. The effect of following housing characteristics on the concentrations of endotoxin and β-D-glucan was investigated: indoor relative humidity, temperature, water intrusion or mold damage on visible surfaces inside homes, presence of cats or dogs, floor type, and number of inhabitants.

Methods

Selection of homes for sampling

This investigation was conducted in 184 homes, selected from a cohort of the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS).[39] Inclusion criteria for homes in this study are described in Reponen *et al.*[40] The University of Cincinnati Institutional Review Board approved the human subject protocol prior to the study initiation. At each home, an informed consent was obtained from the child's primary caregiver.

Home characteristics

For every home, two trained staff members administered a questionnaire and completed home assessment. A validated questionnaire addressed the presence of pets (dog or cat), number of inhabitants currently staying in the home, and history of water damage. Relative humidity and ambient temperature were measured in every room during home assessments. The presence of carpet in each room along with any signs of visible water damage or visible fungal contamination on surfaces inside the house was also noted using a checklist, as previously described.[41] Cumulative areas of water damage were measured using scaled photographs of these areas taken during inspections. Tape samples were collected to confirm suspected fungal growth. Homes were categorized on the basis of parameters such as the reported history or observation of water damage, presence of moldy odor inside homes or visible mold on the surface inside the home. Mold damage in homes was quantified into three categories: Category 0 (no visible mold or moldy odor), Category 1 (low mold: reported history or observed water damage, observed moldy odor, or visible mold area <0.2 m^2), and Category 2 (high mold: visible mold area >0.2 m^2).[40,41]

Microbial contaminants

For every home included in this investigation, sampling of air and floor dust was conducted in the child's primary activity room. The primary activity room was defined as a room in which the participating child spent most of the time while awake.

Air sampling. Two-stage Bioaerosol Cyclone samplers (BC212) designed and supplied by the National Institute for Occupational Safety and Health (NIOSH) were used for collecting particle size-selective air samples defined below.[42] Two conical (1.5 mL) sterile micro-centrifuge tubes and a back-up polycarbonate filter (37 mm diameter, 0.8 μm pore size, Millipore, Billerica, MA) on a polypropylene support were utilized to collect airborne particles in three fractions based on their median aerodynamic diameter: particles with $d_a \geq 1.8$ μm were collected in the first conical tube, those with $d_a = 1.0$–1.8 μm were collected in the second conical tube, and sub-micrometre particles (d_a < 1 μm), referred to as PM1, were captured by the back-up filter. Only the PM1 results were used for the present study. In addition, inhalable particles were collected with a Button Inhalable Aerosol Sampler (SKC,

Inc., Eighty-Four, PA) on a 25 mm polycarbonate membrane filter (3 μm pore size, GE Osmonics, Inc., Minnetonka, MN).

Each sampler was connected to a sampling pump calibrated for a nominal flow rate (3.5 L min^{-1} for BC212 and 4.0 L min^{-1} for the Button Sampler) using a DryCal DC-Lite calibrator (Bios International Corp., Butler, NJ). The sampling flow rate was also verified at the end of the 24 hour sampling period. The average of the pre-sampling and post-sampling flow rate values was used for calculating the concentrations. Concurrent air sampling with the two types of samplers was performed for 24 hours. Sampling strategy, transport and storage of filters are as described by Singh *et al.* (2011).

Dust sampling. Dust sampling protocol used in the CCAAPS has been described by Reponen *et al.*[40] Briefly, an area of 2 m^2 of carpeted floors was vacuumed for 8 minutes using a Filter Queen Majestic® vacuum cleaner (Health-Mor, HMI Industries Inc., Seven Hills, OH). The same vacuum cleaner was deployed in all homes. For rooms without carpet ($n = 42$) the entire floor surface was measured and subsequently vacuumed. Disposable dust filters were used for the sample collection.

The dust samples were sieved through a 355 μm sieve and stored in Pyrogen free tubes at −20 °C until extraction and analysis. To ensure similar handling of analytical protocols and same level of precision in performing analyses, extraction and analysis of the samples were done by the same person. Occupants were requested beforehand not to clean their floors during and at least 24 hours before the collection of air samples. Dust sample was collected immediately after finishing the air sampling to avoid temporary increase in air levels due to aerosolization of dust particles.

Analytical protocols for endotoxin and β-D-glucan assays of air and dust samples

Extraction of air and dust samples: extraction of air sampling filters from NIOSH BC212 and Button sampler and of dust samples have been described in previous studies.[40,43–45]

Endotoxin assay. Endotoxin specific quantitative kinetic chromogenic Limulus Amebocyte Lysate method: pyrochrome (Associates of Cape Cod, East Falmouth, MA) was used for assaying endotoxin from air and dust samples. An automated microtiter plate reader was used with the KC4™ v3.4 software (BIO-TEK Instruments, Inc., VT). The endotoxin concentrations were expressed in EU m^{-3} for air samples and in EU mg^{-1} for dust samples. The LLOD for the endotoxin assay was 0.053 EU mL^{-1}. When taking into account the sampled air volume and the volume of the extraction liquid, this corresponds to LLOD = 0.052 EU m^{-3} for PM1 endotoxin, 0.046 EU m^{-3} for inhalable endotoxin, and 0.002 EU mg^{-1} for dust endotoxin. Of all the PM1 samples, 31% had endotoxin concentrations below the LLOD, whereas about 3% of endotoxin samples for inhalable particles were below the LLOD. None of the dust samples had endotoxin below the LLOD.

β-D-Glucan assay. The assays for β-D-glucan in dust and air samples were performed with (1,3)-β-D-Glucan Detection Reagent Kit: Glucatell (Associates of Cape Cod, East Falmouth, MA) using the kinetic assay. The final β-D-glucan concentration for air samples was expressed in ng m^{-3} and in μg g^{-1} for dust samples. The LLOD for this assay was 2.538 pg mL^{-1}, which corresponds to LLOD = 3 pg m^{-3} for PM1 β-D-glucan, 0.004 ng m^{-3} for β-D-glucan in inhalable particles and 0.0001 μg g^{-1} for dust β-D-glucan concentration. Approximately 18% of all β-D-glucan samples were below the LLOD in PM1 fraction. Two samples had the inhalable β-D-glucan levels below the LLOD. All the dust samples were quantifiable for β-D-glucan.

Additional data analyses were done in this study to assess the associations between microbial contaminants and levels of dog allergen (canf1). Dog allergen levels were determined in dust samples by extracting them from dust using phosphate buffered saline (with Tween 20) and subsequently analyzing these extracts for quantitative estimation using antibody based enzyme linked immunosorbent assay (ELISA).[41]

Statistical methods

Concentrations of endotoxin and β-D-glucan in dust as well as those in airborne particles were tested for normality. The assumption of normality held after log transformation as determined by Shapiro–Wilk tests. Median (minimum, maximum) values of endotoxin and β-D-glucan concentrations were calculated for each exposure variable. The airborne and dust concentrations of endotoxin and β-D-glucan that were below the LLOD were divided by two before subsequent analyses. The hypothetical LLOD for each microbial contaminant in the respective sample was calculated based on a constant nominal sampling flow rate and 24 hour sampling time, although the actual mean sampling flow rates and sampling times varied somewhat for each home. These hypothetical LLODs were considered for censored regression analyses of PM1 particulate concentrations.

Simple Pearson correlation coefficients were calculated for each pair of endotoxin exposure measures, and each pair of β-D-glucan exposure measures (PM1 and inhalable, PM1 and dust, inhalable and dust). General linear regression analyses (Tobit for PM1 particles) were performed to assess associations between microbial contaminants and home characteristics. Tobit regression analyses (left censoring) were performed for PM1 concentrations because more than 15% of these values were below the lower limit of detection. For multivariate analyses, all the home characteristics that were significant in univariate analyses ($p \leq 0.15$) were considered. Those with $p < 0.05$ in multivariate analyses were considered to have significant associations with corresponding exposures. In the correlation analysis, each microbial contaminant was controlled for home characteristics that were significantly related to it in the multivariate analyses, and the Pearson correlations were recalculated for each variable pair using partial correlations (partial for significant home characteristics). Partial and simple correlations of the same pair were compared to explain if differences could be explained by the effect of one or more home characteristics.

Results

Concentrations of microbial contaminants

Table 1 shows descriptive statistics of the concentrations of microbial contaminants. The median values for airborne PM1 endotoxin and β-D-glucan were 0.11 EU m^{-3} and 0.59 ng m^{-3}.

Concentrations of inhalable particles of endotoxin and β-D-glucan were higher, as expected, with median values = 4.28 EU mg^{-1} and 1.96 ng m^{-3}, respectively. There were, however, larger variations in the PM1 concentrations than in the inhalable concentrations of endotoxin and β-D-glucan between different homes. Median values for endotoxin and β-D-glucan concentrations in settled dust were 153.63 EU mg^{-1} and 153.19 μg g^{-1}.

Correlations between microbial contaminants

Table 2 shows simple Pearson correlation coefficients between airborne and dustborne contaminants. In this correlation matrix, the associations between variables were determined without adjusting (simple correlation) for the home characteristics. PM1 endotoxin had significant positive correlation with inhalable endotoxin ($r = 0.33$, $p < 0.001$) and with dust endotoxin concentration ($r = 0.26$, $p < 0.001$). Similarly, inhalable endotoxin had significant positive correlation with dust endotoxin concentration ($r = 0.34$, $p < 0.001$). Between β-D-glucan levels in different exposure matrices, significant correlations were determined between inhalable and dust concentrations only ($r = 0.18$, $p < 0.01$).

Distribution of home characteristics

Summary of home characteristics are shown in Table 3. Of all the homes in the study ($n = 184$) about 77% had carpeted floors in the areas of air and dust sampling. Approximately 21%, 69% and 10% of homes were categorized as mold category '0', '1' and '2', respectively. Among homes with pets, more homes had dog(s) (about 37%) than cat(s) (about 18%). Mean (standard deviation) values of indoor relative humidity and temperature during the visits were 41.1 (11.3) % and 23.8 (2.4) °C. Relatively low but statistically significant seasonal variations of relative humidity (R-square = 0.12; $p < 0.01$) and temperature (R-square = 0.14; $p < 0.01$) were observed (data not shown). Mean (standard deviation) number of inhabitants in these homes was 4.7 (1.4). The concentration of dog allergen in dust samples varied from 242 μg g^{-1} of house dust to below the limit of detection (0.0052 μg g^{-1}).

Associations between concentrations of microbial contaminants and home characteristics

Table 4 shows univariate associations of the microbial contaminants with the home characteristics. Airborne endotoxin levels in PM1 or in inhalable particles were not significantly associated with any of the home characteristics considered in this study. Dust endotoxin concentration significantly increased with increase in relative humidity ($p < 0.05$), increase in temperature ($p < 0.01$) and increase in the number of inhabitants in the home ($p < 0.01$).

Every 5% increase in relative humidity was associated with increased dust endotoxin concentration by a factor of 1.05. This factor was calculated by exponentiation of five times the parameter estimates of the independent variables ($e^{5 \times 0.01}$, where 0.01 is the parameter estimate obtained for relative humidity, see Table 4).

Airborne PM1 and inhalable β-D-glucan levels were significantly associated with relative humidity ($p < 0.05$ for both). Dust β-D-glucan concentrations were significantly lower in homes with one or more dogs ($p < 0.05$) and in those with carpeted areas ($p < 0.01$). Mold category classification between homes or the presence of cats was not significantly associated with any of the microbial contaminants examined in this study. Additional analysis determined a significant negative association between dog allergen and β-D-glucan concentration in dust samples (estimates −0.05, $p < 0.05$) (data not shown). Season of sampling

Table 1 Median (minimum, maximum) concentrations of microbial contaminants in 184 homes

Exposures	Media	Dependent variables	Median (minimum, maximum)
Endotoxin	Air	PM1	0.11 (0.05, 11.14) EU m^{-3}
		Inhalable	4.28 (0.02, 389.17) EU m^{-3}
	Dust	Concentration	153.63 (12.09, 1.06×10^4) EU mg^{-1}
β-D-Glucan	Air	PM1	0.59 (0.05, 29.05) ng m^{-3}
		Inhalable	1.96 (0.002, 41.91) ng m^{-3}
	Dust	Concentration	153.19 (1.32, 3204) ng g^{-1}

Table 2 Pearson correlation coefficients (unadjusted) between endotoxin and β-D-glucan levels in air (PM1, inhalable) and dust[a]

		Air	
		PM1	Inhalable
Endotoxin			
Air	Inhalable	**0.33*****	
Dust	Concentration	**0.26*****	**0.34*****
β-D-Glucan			
Air	Inhalable	0.04	
Dust	Concentration	0.06	**0.18****

[a] Note: $\log_e$ transformed values of endotoxin and β-D-glucan were correlated without adjustment for different home characteristics. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Significance levels were $p \geq 0.05$ for all other correlations. Significant correlations are shown in bold. (PM1 = particulate matter with $d_{ae} < 1.0$ μm).

Table 3 Descriptive statistics of home characteristics: mean values (standard deviation) for continuous variables and percentages in different categories for categorical variables

Home characteristics	
Continuous variables	Mean (standard deviation)
Relative humidity (%)	41.06 (11.29)
Temperature/°C	23.75 (2.44)
Number of inhabitants	4.67 (1.37)
Categorical variables	*n* (%)
Carpet	
Yes	142 (77.2)
No	42 (22.8)
Mold category	
0	38 (20.7)
1	127 (69.0)
2	19 (10.3)
Dog(s)	
No	116 (63.0)
Yes	68 (37.0)
Cat(s)	
No	151 (82.1)
Yes	33 (17.9)

Table 4 Univariate associations between concentrations of microbial contaminants and home characteristics[a]

		Endotoxin		β-D-Glucan	
Media	Home characteristics (independent variables)	Effect size	*p*-Value	Effect size	*p*-Value
PM1	Dog(s) (yes)	0.95	0.12	1.38	0.14
	Cat(s) (yes)	0.49	0.65	−0.77	0.48
	Carpet (yes)	0.18	0.77	−0.10	0.94
	Relative humidity	0.04	0.09	**0.08**	**0.03**
	Temperature	−0.10	0.68	0.20	0.18
	Mold category		0.26		0.88
	2	−1.09		−0.77	
	1	−0.12		0.01	
	# Inhabitants	−0.58		0.52	0.09
Inhalable	Dog(s) (yes)	−0.12	0.82	−0.52	0.23
	Cat(s) (yes)	0.54	0.41	0.58	0.33
	Carpet (yes)	0.61	0.60	0.26	0.67
	Relative humidity	0.01	0.76	**0.06**	**0.02**
	Temperature	−0.18	0.08	0.14	0.18
	Mold category	0.37	0.95		0.77
	2	0.37		−0.26	
	1	−0.06		−0.32	
	# Inhabitants	0.35	0.46	0.10	0.70
Dust concentration	Dog(s) (yes)	0.16	0.75	**−0.90**	**0.02**
	Cat(s) (yes)	0.70	0.09	0.28	0.59
	Carpet (yes)	0.61	0.49	**−1.58**	**<0.01**
	Relative humidity	**0.01**	**0.02**	0.01	0.64
	Temperature	**0.18**	**<0.01**	−0.04	0.72
	Mold category		0.61		0.47
	2	0.20		0.08	
	1	−0.16		0.52	
	# Inhabitants	**1.75**	**0.01**	0.49	0.24

[a] Effect size is equal to the change in the dependent variable corresponding to a unit increase in the independent variable. (Note: values for significant associations ($p < 0.05$) are shown in bold).

had significant influence on the airborne inhalable endotoxin concentrations: the concentrations were highest in spring and lowest in summer ($p < 0.01$). Other exposure variables were not significantly affected by season.

Table 5 presents the results of multiple regression analyses on the association between microbial contaminants and home characteristics. Similar to the univariate analysis, associations of endotoxin concentration in PM1 or inhalable particles with the home characteristics were not significant ($p > 0.05$) in the final multivariate regression models.

Dust endotoxin concentration increased significantly with increasing temperature ($p < 0.01$) and the number of inhabitants ($p < 0.01$).

Although relative humidity was significantly associated with PM1 β-D-glucan concentration in the univariate analysis, this association was not significant when multiple home characteristics were considered together. Inhalable β-D-glucan concentrations, however, had significant positive association with relative humidity ($p < 0.05$) in the multivariate analyses. Presence of dog (s) ($p < 0.05$) and carpeted floor ($p < 0.01$) was significantly associated with decrease in the dust β-D-glucan concentration. Adjusting for the home characteristics did not significantly influence the correlations between the concentrations of microbial contaminants. For example, the adjusted Pearson correlation coefficients (*p*-value) between inhalable and dust β-D-glucan concentrations increased from 0.18 to 0.22 ($p < 0.01$ for both adjusted and unadjusted correlations).

Discussion

Correlations between airborne and dustborne microbial contaminants

Statistically significant correlations were found between airborne and dustborne microbial contaminant levels, and these correlations were stronger between airborne and dustborne endotoxin concentrations than with respective β-D-glucan concentrations. Similar to our results, other studies found statistically significant, but weak correlations between airborne and dustborne microbial concentrations.[13,25] Microbial contaminants found in house dust could have been transferred from outside by foot traffic but may not have been aerosolized and thus their levels may represent different exposure pathways.[40] Indoor airborne levels of microbial contaminants could vary widely and may be affected by reaerosolization from surface dust or from movement of these particles from outside sources.[3] We used long-term sampling (24 hours) to overcome these limitations in air sampling.

These significant correlations have important implications for understanding the actual exposure. Statistically significant relationships even with low correlation coefficients would assist in uncovering relationships between the variables. In our study, the strongest correlation between airborne and dustborne contaminants was found between inhalable endotoxin and dust endotoxin with $r = 0.34$. This suggests that 12% (r^2) of the variability in inhalable endotoxin can be explained by dust endotoxin levels. To predict the levels of one variable from the related variable,

Table 5 Multivariate association between concentrations of microbial contaminants and home characteristics

Exposure (dependent variable)		Home characteristics (independent variable)	Effect size[a] (95% confidence intervals)
Air endotoxin	PM1	Relative humidity	1.55 (−0.40, 3.51)
		Dogs (yes)	1.39 (−0.57, 3.35)
	Inhalable	Temperature	−1.78 (−3.75, 0.20)
Dust endotoxin concentration		Temperature	**2.87** (0.89, 4.84)**
		# Inhabitants	**2.76** (0.79, 4.74)**
Air β-D-glucan	PM1	Relative humidity	1.87 (−0.10, 3.83)
		# Inhabitants	1.49 (−0.47, 3.45)
		Dogs (yes)	1.17 (−0.32 1.29)
	Inhalable	Relative humidity	**2.32* (0.34, 4.29)**
Dust β-D-glucan concentration		Dogs (yes)	**−2.24* (−4.12, −0.24)**
		Carpet (+)	**−3.05** (−5.03, −1.09)**
		# Inhabitants	1.45 (−0.52, 3.42)

[a] Effect size is equal to the change in the dependent variable corresponding to a unit increase in the independent variable. Note: initial model included covariates, which were significant at the 15% level in univariate regression models. The covariates included: relative humidity, temperature, number of inhabitants, dog(s)/cat(s) in home, carpet in homes and mold category of homes. * $p < 0.05$; ** $p < 0.01$. Significant associations are shown in bold.

however, a statistically significant higher coefficient ($r \geq 0.9$) between the two variables is required.[46,47] Based on this, the statistical significance suggests an association between airborne and dustborne microbial concentration, but does not warrant the prediction of airborne concentration from the dustborne concentration and *vice versa*.

While considering the effect of airborne particle size on correlations of microbial contaminants in air and dust, stronger correlations were observed between inhalable and dust contaminants than between PM1 and dust contaminants, which can be explained by gravitational settling and reaerosolization of particles. Inhalable fraction is represented by much larger particle sizes, which settle faster and are easier to reaerosolize from surfaces than PM1. Interestingly, adjusting for home characteristics, such as number of inhabitants, did not essentially change the associations between dustborne and airborne contaminants. This indicates that factors affecting microbial growth in dust may differ from those affecting aerosolization of microorganisms or their cellular components. Thus, a variety of factors inside homes and those inherent to particles influence microbial particulate concentrations in dust and in air.

Among the airborne microbial contaminants, stronger association between PM1 and inhalable concentrations was found for endotoxin than for β-D-glucan. As described in the following discussion this may be related to the differences in the size of intact bacterial cells and fungal spores. The prevalence of specific bacterial genera in dust or airborne samples collected inside homes was not assessed in this study. However, Gram-negative bacteria frequently found in home environments are *Pseudomonas* and *Aerobacter*, which vary in size from <1 μm to about 7 μm.[48–51] Intact bacteria can, therefore be found both in PM1 and inhalable particles.[7] Aerodynamic size of airborne fungal spores is generally above 2 μm[49,52] Thus, in contrast to endotoxin, β-D-glucan in PM1 consists of microbial fragments which may have a different aerosolization pattern than intact fungal spores. Findings in the current study support our previously reported data obtained in a different set of homes where β-D-glucan concentration in PM1 size fraction did not correlate significantly with that in larger particles ($d_a > 2.25$ μm).[4]

Associations between home characteristics and concentrations of microbial contaminants

Relative humidity and temperature. Although several significant associations were observed between the concentrations of microbial contaminants and temperature and relative humidity in the univariate analyses, most of the associations disappeared in the multivariate analysis. This indicates that other factors besides relative humidity might be affecting the concentrations of microbial contaminants in homes.[53–55] The effects of such characteristics on the concentrations could be counteractive to the effects of relative humidity, which might weaken these associations.

Relative humidity remained significantly associated only with inhalable β-D-glucan in the multivariate analyses. Higher humidity could favor agglomeration of airborne particles, and hence increase the course particle fraction (covering the inhalable size range).[56] Interestingly, β-D-glucan concentration in dust was not significantly associated with relative humidity. Fungal genera commonly detected in air samples taken in this study were *Aspergillus*/*Penicillium*, Ascospores and *Cladosporium*.[45] These fungi require a high relative humidity of about 50–70% for their optimal growth and proliferation.[37] The comparatively lower mean relative humidity of 41.1% inside the homes of this study suggests that although these genera would have survived on the surfaces, the indoor humidity would not have been very conducive to their proliferation. Moreover, humidity on the surface where these fungi grow would be more important for their survival and proliferation than ambient air humidity.

Another significant association in the multivariate model was seen between dustborne endotoxin concentration and temperature. This can be attributed to the fact that bacterial cells require appropriate temperature for multiplication. Cell duplication increases with increase in temperature with cell cycle duration being least at a temperature of about 30 °C for most bacteria.[57] The mean indoor temperature of approximately 24 °C measured in our study homes appears favorable, even if not optimum, for bacterial growth. Because relative humidity and temperature were significantly different between seasons, these variables were considered as surrogates for different seasons. Therefore, season was not considered in the multivariate models for predicting concentrations of these exposure variables in homes.

Other home characteristics. Number of inhabitants, presence of dogs and carpet in home were associated with the

concentrations of dustborne but not with airborne contaminants. Dust endotoxin levels increased with the number of inhabitants. This supports the results of other studies[17,20,58] and can be explained by occupants being the direct source of endotoxin. Taubel *et al.* (2007) found Gram-negative bacteria, mostly Proteobacteria, in wipe samples collected from human skin.[59]

Dust β-D-glucan concentrations significantly decreased with the presence of dogs and carpeted floors. Chi-square tests between the number of homes with or without dogs and carpets revealed no significant differences. Thus, it is evident that carpeted floors and the presence of dogs independently had significant influence in having lower β-D-glucan concentration in settled floor dust. The negative association between the presence of dogs and dust β-D-glucan levels seems elusive. There might possibly be some other factor(s) for which the presence of dogs served as a surrogate, which influenced dust β-D-glucan concentration. Therefore, the influence of dog allergen on dust β-D-glucan concentration was explored. A significant negative association was detected between these variables in multivariate analyses when adjusted for floor type (carpet/non-carpet). A possible mechanism could be a specific cross-reaction of cellulose components in β-D-glucan with carbohydrate-binding module on these allergens.[60] Thereby the biological activity of β-D-glucan in dust particles might be reduced because of these cross reactions. Another possible explanation is that the presence of dogs could favor physical transport of inert dust particles from outdoors, which could dilute the concentration of microbial contaminants in indoor dust. Presence of carpets, in addition, may retain dog dander better than non-carpeted floor. Consequently, the combined effects of carpeted floor and dog allergen could significantly reduce dust β-D-glucan concentration.

Although the above mentioned associations were observed between dust β-D-glucan and the presence of dogs in homes with carpets, such associations of independent variables were not identified with airborne β-D-glucan. Because of the large size of dog allergens[61] they do not remain aerosolized long enough to exhibit interaction with airborne β-D-glucan.

There was no significant association of the home characteristics with the concentrations of PM1 and inhalable microbial contaminants (except for the positive association between relative humidity and inhalable β-D-glucan). Aerosolization of house dust by human activities could be a major source of airborne dust particles. The lack of association of the airborne levels of microbial contaminants with the number of occupants implies that other factors influence their dispersal or re-suspension more strongly than the occupants. This finding is in agreement with two previous studies conducted in the USA[13,62] but is contradictory to another study conducted in France.[18] The disagreement may be attributed to differences in the occupant density and the type of ventilation used in the homes tested in the above-quoted American and French studies.

In our investigation, presence of cats or the mold category in homes was not significantly associated with the levels of any of the exposure variables. Platts-Mills *et al.* (2001) also did not find an association between the presence of at least one pet (dog or cat) and airborne endotoxin level, although households with cat (s) had significantly lower levels of airborne endotoxins compared to those with dog(s).[19] The lack of association between visible mold and the concentrations of microbial contaminants is consistent with several other studies which did not find any association[25,62,63] or only a borderline significant association[64–66] with concentrations of microbial contaminants.

Limitations

PM1 contaminants were sampled using a bioaerosol cyclone sampler. As with any cyclone sampler there could be particle bounce (resulting primarily from overloading). This could lead to an overestimation of the concentration of contaminants measured in the PM1 fraction. Although this overestimation was not significant in a previous study[45] further studies need to pay attention to the possibility of particle bounce. Moreover, turbulence inside first and second stage collection tubes of the bioaerosol sampler could possibly fragment the larger particles into sub-micrometre particles; as a result the concentrations of microbial contaminants in PM1 could be overestimated. Although there is no direct evidence to substantiate the assumption about particle fragmentation inside these samplers, fragmentation of liquid droplets has been reported in highly turbulent flows as velocity variations exert different dynamic stresses at different points on the particle surface.[67]

Other significant factors, not assessed in this study, that could have affected the airborne concentrations of endotoxin and β-D-glucan are their concentrations in outdoor air and air currents produced by ventilation inside rooms, either natural or from heating, ventilation and air-conditioning (HVAC) systems.[68] The air currents could affect aerosolization of particles from settled dust as well as their transport from one area to another.

Conclusion

Dustborne contaminants were more strongly affected by home characteristics than airborne contaminants. The positive associations between dustborne endotoxin and temperature as well as the number of inhabitants support previous findings. A negative association was found between dust β-D-glucan and presence of dogs and carpet in homes. This was explained by the inhibitory effect of dog allergen on the β-D-glucan assay and the retention of dog allergen by carpets. The correlations between airborne and dustborne contaminants were weak. This indicates that airborne concentrations cannot be reliably predicted based on dustborne concentrations. The lack of correlation between PM1 and inhalable β-D-glucan emphasizes the importance of conducting size-selective air sampling when assessing exposure to β-D-glucan.

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